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Multiple Epiphyseal Dysplasia, Ribbing Type: A Novel Point Mutation in the COMP Gene in a South African Family

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Multiple epiphyseal dysplasia is broadly categorised into the more severe Fairbank and the milder Ribbing types. In this paper we document mild MED in a South African kindred, and demonstrate that heterozygosity for a mutation in the cartilage oligomeric matrix protein (COMP) gene causes the condition. The mutation, C1594G, implies a N523K substitution, altering a residue at the carboxyl-terminal end of the calmodulin-like region of COMP. The identification of this mutation demonstrates that the spectrum of manifestations from mild MED through pseudoachondroplasia can all be produced by structural mutations in COMP. Am. J. Med. Genet. 68:396–400, 1997.

KEY WORDS: cartilage oligomeric matrix protein (COMP); epiphyseal dysplasia; genetic; osteochondrodysplasia

INTRODUCTION

Multiple epiphyseal dysplasia (MED) is an autosomal dominant osteochondrodysplasia characterised by generalized abnormalities of the epiphyses. The severe Fairbank type [Fairbank, 1946] and the milder Ribbing type [Ribbing, 1937] are the two recognised forms. Linkage has been established in affected kindreds to either the EDM1 locus on the proximal short arm of chromosome 19 [Oehlmann et al., 1994; Knowlton et al., 1995] spanning the gene for cartilage oligomeric matrix protein (COMP) [Newton et al., 1994; Briggs et al., 1995], or to the EDM2 locus in the region of chromosome 1 [Briggs et al., 1994], which contains the COL9A2 gene. In another kindred the MED phenotype is linked to neither of these loci [Deere et al., 1995] and it is evident that there is additional non-allelic heterogeneity.

We have investigated a South African family of Western European stock with a mild form of MED. To determine if a mutation at the COMP locus could produce a mild phenotype, we have undertaken SSCP analysis to screen exons of the COMP gene in this family. We demonstrate that the disorder in the kindred is produced by a mutation in exon 14 of the gene.

MATERIALS AND METHODS

The Affected Kindred

The individuals whom we studied were South Africans of Western European stock. The affected persons in the kindred, the proposita, her son, brother, and deceased mother were shorter in height (154 cm, 156 cm, 157 cm, and 152 cm, respectively) than the unaffected relatives who were of normal stature for their population group, but in every instance their habitus was proportionate. Joint pain, mainly in the hips but also in the other large joints were the predominant clinical problem, and three affected individuals had received bilateral prosthetic hip replacements in adulthood. There was some limitation in the range of motion in the affected joints, but articular mobility was otherwise unremarkable. All affected relatives had noticeably shortened digits.

Molecular Investigations

Linkage analysis. The linkage study involved examination of intragenic and flanking markers from the EDM1 [Briggs et al., 1995] and EDM2 [Hellsten et al., 1995; Warman et al., 1994] loci, and markers for the

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COL9A1 locus [Warman et al., 1993]. Two point LOD scores were calculated using the computer programme MLINK version 5.03 [Lathrop and Lalouel, 1984]. The MED gene was assumed to be autosomal dominant with a penetrance of 100% and with an allele frequency of 0.001.

SSCP and sequence analysis. Single strand conformational polymorphisms (SSCPs) were analysed in a non-denaturing 0.5 × MDE gel matrix (AT Biochem) as suggested by the manufacturer. Electrophoresis was performed at 4°C at 3 W for 16 hours and the DNA banding patterns were visualised by silver staining [Lohmann et al., 1992].

Single stranded variant and normal alleles were excised from the SSCP gels and used as templates in PCR reactions. The product was purified (QIAquick, Qiagen) and sequenced manually using the Sequenase version 2.0 DNA sequencing kit (USB).

RESULTS

Radiological Findings

Radiographs were available on the proposita and her affected son. These documented significant flattening and irregularity of femoral heads, with some joint space narrowing and periarticular sclerosis, which was indicative of secondary generalized osteoarthropathy (Fig. 1). The intra-articular aspects of the distal end of the femora and proximal end of the tibiae were uneven and the lateral femoral condyles were hypoplastic (Fig. 2).

The end plates of the vertebral bodies showed mild sclerosis and irregularity but there was no significant flattening. The skeletons were otherwise radiologically normal and in particular, there were no changes in the diaphyses or metaphyses of the tubular bones. Therefore, the diagnoses of pseudoachondroplasia or spondyloepiphyseal dysplasia could be definitely discounted.

Fig. 2. Antero-posterior radiograph of the knees of RP at 21 years. The articular regions of the lower end of the femur and the upper end of the tibia are misshapen and the lateral condyles are hypoplastic.

In the hands, the phalanges were mildly short but not otherwise dysplastic (Fig. 3). Pattern profile analysis, using the method of Poznanski et al. [1972], showed that these changes were maximal in the metacarpals, with a trend to lesser severity in the distal phalanges (Fig. 4).

Molecular Findings

The structure of the family under investigation is shown in the pedigree in Figure 5, and DNA from individuals II-3, II-4, II-5, II-6, III-4, III-5, and III-6

Fig. 1. Antero-posterior radiograph of the pelvis of the proposita at the age of 45 years. The femoral head on the right side is irregular and somewhat flattened. Areas of patchy sclerosis and lucency are evident. Prosthetic joint replacement has been undertaken on the left side.

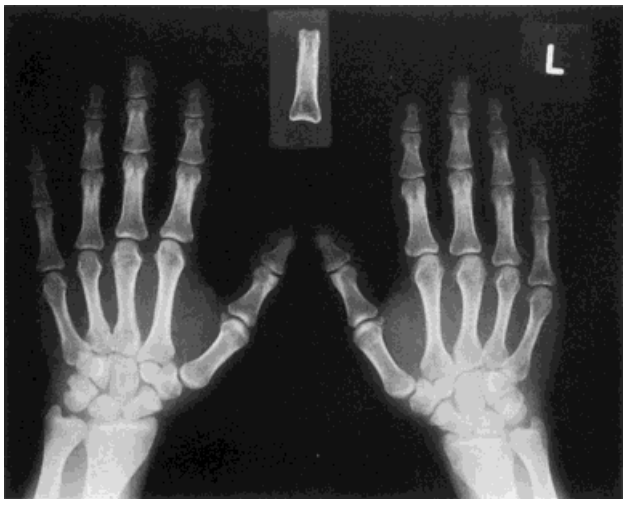

Fig. 3. Antero-posterior radiographs of the hands of the proposita's son at the age of 21 years. The tubular bones are shortened but not dysplastic.

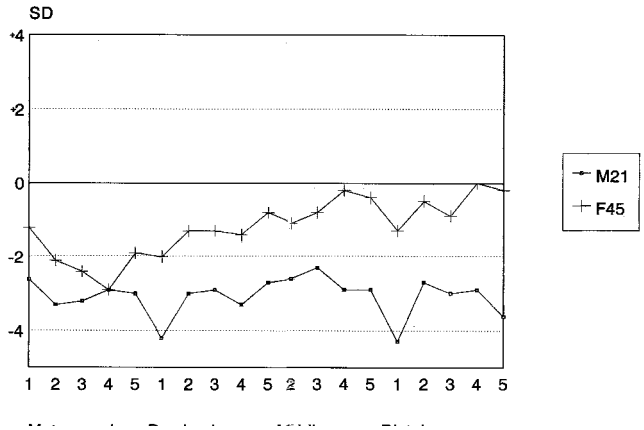

Fig. 4. Pattern profile analysis of the digits of the proposita age 45 (F45) and her son, age 21 (M21). Both have shortness of all tubular bones with a minor trend towards decrease in severity from the metacarpals to the distal phalanges.

was available for linkage analysis. A LOD score of −5.52 at theta=0.00, using a marker from the closely linked L-myc region on chromosome 1 led to the exclusion of the EDM2 candidate locus. An inheritance pattern consistent with linkage was found for the markers for the COL9A1 and EDM1 loci. The highest observed LOD scores were 0.78 (D19S602 at EDM1) and 0.81 (509-8B2 and 509-12B1 at COL9A1) at theta=0.00.

Because of evidence of linkage between the chromosome 19 markers and the disease phenotype in the South African MED family, exons 9–14 of the COMP gene were screened using SSCP analysis. A conformational polymorphism in an amplified DNA fragment containing exon 14 of the COMP gene cosegregated with the condition in the family without exception (Fig. 5). Neither the unaffected relatives nor 50 controls from the normal South African community of Western European origin, exhibited this SSCP pattern.

DNA sequencing revealed a C to G transition at nucleotide position 1 594 (Fig. 6) and implied substitution of an uncharged polar asparagine residue by a charged lysine residue at position 523 of the COMP protein.

DISCUSSION

The observation that the ultrastructural changes of cartilage in some cases of the Fairbank form of MED and in pseudoachondroplasia are similar [Stanescu et al. 1993] led to the hypothesis that these disorders might be allelic. Thereafter, Oehlmann et al. [1994] localised a MED gene to chromosome 19, while Briggs et al. [1993] showed that the pseudoachondroplasia gene was in the same chromosomal region. However, there is locus heterogeneity as a second MED gene (EDM2) is localised to the region of chromosome 1 containing the COL9A2 gene [Briggs et al., 1994] and a mutation has been identified in another family [Muragaki et al., 1996]. There is further heterogeneity as exclusion of linkage of the MED phenotype has been demonstrated in other families [Deere et al., 1995; MDB and DHC, unpublished data]. Recently, the *COMP* gene, which encodes the cartilage oligomeric matrix protein (COMP), was identified, characterised, and localized to the critical interval for EDM1 [Newton et al., 1994; Briggs et al., 1995]. COMP is a 524 kDa pentameric glycoprotein of the cartilage extracellular matrix and is a member of the thrombospondin family of genes [Bornstein et al., 1993].

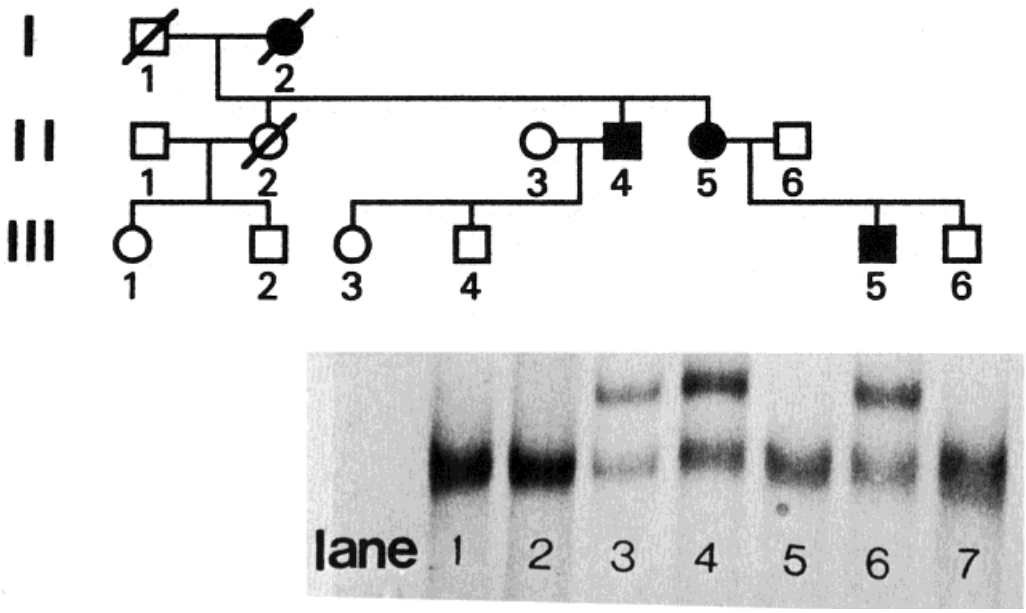

Fig. 5. Pedigree and PCR-SSCP analysis of exon 14 of the COMP gene in a South African MED kindred. Shaded symbols on the pedigree represent affected individuals. The SSCP change cosegregates with the disorder in the family.

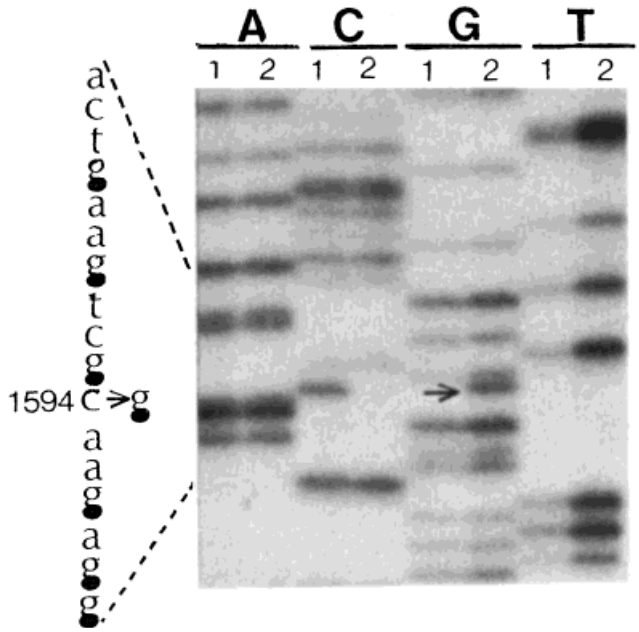

Fig. 6. Illustration of the sequence of the region within exon 14 of the COMP gene, containing the C→G transition at nt position 1594.

It was demonstrated by Briggs et al. [1995] that mutations in the COMP gene, in the region that encodes a putative Ca++ binding motif, were responsible for MED type Fairbank and pseudoachondroplasia. In addition, Hecht et al. [1995] independently identified mutations in COMP, which caused pseudoachondroplasia. These studies showed that alterations of conserved aspartic acid and cysteine residues, which are thought to maintain the structure and integrity of the calcium binding domain, produce these phenotypes. In the South African kindred reported here, the Asn523Lys substitution, while not in an evolutionarily conserved position, is located immediately adjacent to a highly positionally conserved aspartic acid residue within the eighth calmodulin-like repeat of the molecule. Assuming that, like the aspartic acid residues in calmodulin, this residue is involved in the coordination of calcium ions, a change in the local ionic environment by the substitution of a larger, charged lysine residue might reduce the charge-charge interactions, which usually occur between Ca++ ions and the receptive amino acids in this region. As a consequence, the structural integrity of the COMP monomer may be compromised and/or an effect on calcium-dependent binding between COMP and other components of the extracellular matrix may result.

Owing to the informativeness of the markers and the small family size, it is not surprising that an inheritance pattern consistent with linkage to more than one locus was observed. This finding highlights the potential uncertainty in using just linkage analysis in diagnostic studies in small families with a heterogeneous disorder and argues that the identification of a mutation is necessary to confirm the association between the gene and the condition.

It is of interest that allelic mutations in COMP have been described in patients with the MED Fairbank and pseudoachondroplasia phenotypes. The clinical and radiographic manifestations of these two phenotypes are distinct, and diagnostic differentiation between the disorders is not difficult. However, it is becoming clear that mutations in COMP unify phenotypes that span a phenotypic spectrum and, between the extremes, the phenotypic distinction may not be entirely clear cut; for instance, some patients with MED have mild changes in the metaphyses and vertebrae which could be regarded as a continuum with pseudoachondroplasia. The phenotype of the South African family described here approximates the mild Ribbing type of MED. The identification of a mutation in the COMP gene in this family expands the range of phenotypes that can be produced by COMP mutations, defining a spectrum of allelic disorders from severe pseudoachondroplasia through mild MED.

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